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ORIGINAL ARTICLE

To Evaluate the Toxic Effects of Lithium Carbonate on Granule Cells Count of Rat Cerebellum

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ABSTRACT

Aim: To study the damaging effect of chronic ingestion of 20 mg/kg body weight/OD of lithium carbonate on cerebellargranule cells.
Methods: However, there is scanty documented information about the cerebellar toxicities of lithium carbonate on granule neurons. Therefore the present study is designed to observe the microscopic changes of granule neurons in rat cerebellum. For this experimental study 20 animals were used, they were divided into two groups, each comprising of 10 animals.
Results: Group-A received normal lab diet and water ad libitum while group B received lithium carbonate 20 mg/kg/OD for 2 weeks and 6 weeks respectively. Micrometry was done on granule cells count.
Conclusion: Highly significant changes of granule cells count were observed even at therapeutic doses. Lithium carbonate causes oxidant injury to granule neuronal cells in rat cerebellum.
Keywords: Oxidant injury, Cerebellar degeneration, Incoordination,

INTRODUCTION

Literature supports Lithium, as thesuccessful drug,for depression[1].Research conducted by de Cerqueira et al[2,3] (2008) had reported that lithium causes cerebellar degeneration due to significant neuronal loss. At therapeutic doses the most common adverse effects like tremors, incoordination[4]were reported.

MATERIALS AND METHODS

This study was carried out during the period from April to June 2012. For this experimental study 20 albino rats weighing 140 – 170 grams were selected. They were obtained from Charles River Breeding Laboratories, Brooklyn, Massachusetts, USA, and were cross bred at Animal House of Basic Medical Sciences Institute, JPMC, and Karachi. The animals were kept in Animal House on a balanced diet. They were put under observation for one week prior to the experiment.

The animals were divided into two groups a control group-A (n=10) and lithium carbonate group-B (n=10). The rats in control group-A were given labdiet and water by feeding tube for a time period of 2 and 6 weeks while the rats in group-B received lithium carbonate in powder form mixed in flour pellets at a dose of 20 mg/kg body weight/OD for 2 and 6 weeks respectively[5].

On day 14 and 42 the animals were sacrificed, brain was removed; the cerebellum was separated from the rest of the brain and fixed in formaldehyde [7] for 24 hours.

The cerebellar tissue was dehydrated by passing through ascending grades of alcohol cleared by xylene and infiltrated by paraffin. The fixed tissue blocks were sectioned and obtained on glass slides four micron thick sections were collected for staining with haematoxylin and eosin[8] (Bancroft and Cook, 1984).

The changes of the granule cells count were observed under light microscope. Observations were recorded for granule cells count in each group according to time interval. The cells count was made fewer than40 x objectives with reticule in selected fields of the tissue. The data was subjected to statistical analysis by using software SPSS (Statistical Program for Social Sciences) 2007 version-16. A statistical difference between means and experimental data was carried out by student 'T' test.

Major Group	2nd Week	6th Week
A vs B	P<0.001****	P<0.001****
Key: **** Highly significant		

Statistical analysis of cerebellar granules cells count of major group-B (Lithium carbonate treated) shows a highly significant decrease cell count at 2 and 6 weeks time interval as compared to the major group-A (control)

RESULTS

Group-A1 and B1 (2 weeks). On histological examination of H&E stained section of the gray matter showed multiple basophilic cerebellar granule cells in the granule cell layer.

A highly significant (P<0.001) decreased in the mean values of the granule cells count was observed in the granule cells count of group B1 (57.8±0.09) (Cells/μm)^2 when compared with A1 (67.8±0.24) (Cells/μm)
A highly significantly (P<0.001) decreased in the mean values of the granule cells count was observed in group B2 (49.2±0.23) (Cells/μm)^2 was observed when compared with A2 (68.4±0.19) (Cells/μm)^2. Group-A2 and B2 (6 weeks)

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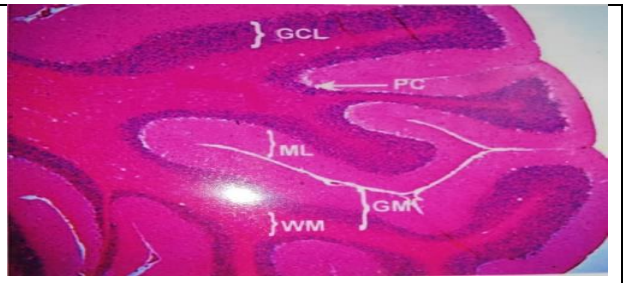
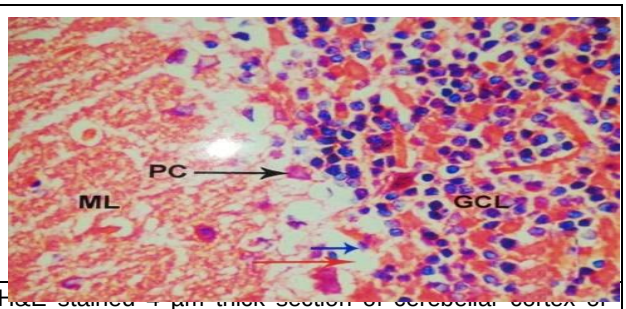
Table 1: Comparison of the mean values of cerebellar granule cells count (Cells/µm)^{2}among Groups-A and B albino rats according to time interval

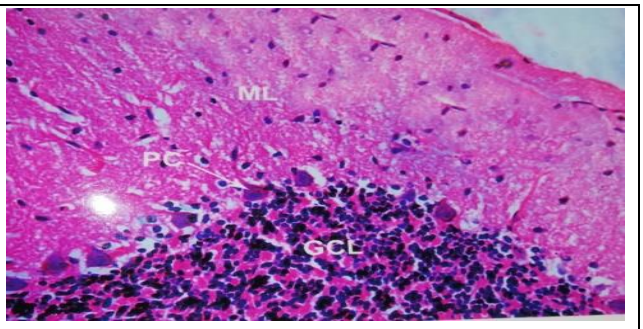
Major Groups	No. of Subjects	2nd Week (Group-A1)		6th Week (Group-A2)		P Value
		Mean	SEM	Mean	SEM	2nd week vs 6th week
A Normal Diet	10	67.8	0.24	68.4	0.19	0.10
B Normal Diet + Lithium Carbonate	10	57.8	0.09	49.2	0.23	0.001



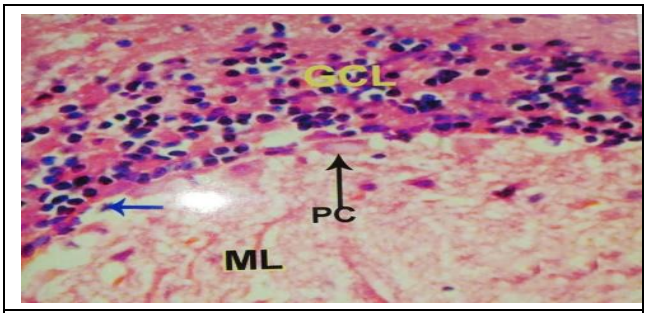
H&E stained 4 µm thick section of cerebellar cortex of group-A1 rat shows normal morphology and normal granule cells count of cerebellar cortex in albino rats.



H&E stained 4 µm thick section of cerebellar cortex of group-B1 rat shows decreased granule cells count of cerebellar cortex in albino rats.



H&E stained 4 µm thick section of group-A2 rats cerebellar cortex shows a highly significant increase granule cells count.



H&E stained 4 µm thick section of group-B2 rats cerebellar cortex shows a highly significant decrease granule cells count.

DISCUSSION

Lithium carbonate (Li2CO3)[9] is the recommended mood stabilizing[10]drug and efficientlyused in the known casesof mood disorder[11].Lithium is frequently used in Depression which is the important cornerstone leading tosuicide[12].

KC Whitley (et al 2020) in their study observed that lithium administration caused glycogen synthase kinase (GSK) inhibition. Glycogen synthetase kinase 3 phosphorylates causes critical cellular processes. Many of these processes, such as autophagy, cell survival/differentiation, and cell cycle are affected due to lithium ingestionandthisresults in the pathogenesis of human neuronal disease due to inhibition of glycogen.[13]This has consequently led toevidence that lithium increased translocation of nuclear factors of activated T cell (NFAT)leading to increased Fas ligand, which led to apoptosis by activation of caspases-3.Increase in the concentration of Caspases -3causes neuronal cellular degradation and the levels of lithium induced apoptosis were highest in the rat cerebellum as reported by Gomez-Sintes and Lucas (2010)[14]. Human central nervous system, has limited capacity, ofrestoration. In the new millennium drug therapies have evolved, to limit the damage through novel therapeutic strategie (Friedlander, 2003)[15]. Evidence suggests, that caspases-3 is a key enzyme, in neuronal apoptosis which is inhibited by Methylcobalamin therapy decreasing cell damage (Birch et al., 2009)[16].

In our study it was also observed that there is decrease in granule cells count in Lithium treated animals. This is also in agreed by Isaev NK et al (1996)[17] ,who in their study showed that glutamate treatment caused clumping of chromatin and disruption of mitochondria due to calcium overload of the neurons which leads to neuronal death.

In the light of above consideration Lithium carbonate proved to be neurotoxic at a therapeutic dose of 20 mg/kg body weight/OD, so the detrimental effect of Lithium carbonate needs special caution for human subjects.

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